Laboratory of Environmental Toxicology:
Summary Statement

by R. L. Dixon

The scientific efforts of the Laboratory of Environmental Toxicology (LET) are directed toward the prevention of environmentally related diseases through understanding mechanisms of toxicity. Special emphasis is placed upon environmental factors which may adversely affect reproduction and development. Toxicologic phenomena are studied from the descriptive to the molecular level in search of a better understanding of mechanisms. This increased understanding contributes to the development of better and more effective test procedures to predict toxicity in humans. Available scientific expertise is broad and includes biochemistry, reproductive physiology, teratology, pharmacology, cell biology, pathology, and behavior. Test systems are likewise diverse and range from molecular biochemistry and electron microscopy, through *in vitro* cell, organ, and embryo cultures, to whole animals. Whether their study be molecular and/or biochemical mechanisms of toxic action, pharmacology and pharmacokinetics in developing systems, the identification of sensitive biochemical indicators which predict specific organ damage, or some other area of toxicology, scientists in LET are in a unique position to develop and utilize basic scientific information for resolving critical environmental health problems.

The research goals of the Laboratory are supported by both intramural scientists and contracted research. The scope of the overall effort is broad involving both national and international programs and adjunct appointments. Recognizing training as one of its primary responsibilities, the Laboratory supports graduate students at nearby universities, offers training opportunities for foreign postdoctoral scientists and American scientists on sabbatical leave. In short, the Laboratory of Environmental Toxicology integrates research and training into a program that is both worldwide in scope and one of the strongest multidisciplinary efforts in toxicology in this country.

Experimental Teratogenesis

The research of this group seeks a better understanding of teratogenic compounds, particularly their molecular mechanisms of action. Scientists are currently testing the hypothesis that certain drugs and environmental chemicals interfere with embryogenesis by irreversibly combining with critical molecules in the fetus. As a first step to investigate this possibility, known acylating agents of simple chemical structure, including anhydrides and imides, are being used as test compounds. To determine the teratogenicity and embryotoxicity of these agents, each is administered to pregnant CD-1 mice. One day prior to full term, fetuses are removed and examined for gross visceral or skeletal abnormalities. All of the acylating agents thus far tested have produced malformations in the offspring of treated dams.

In order to correlate teratogenic potential with chemical reactivity, the interaction of acylating agents with fetal and maternal macromolecules *in vivo* and proteins and model nucleophiles *in vitro* is being studied. Following the administration of ^{14}C phthalic anhydride, covalently bound radioactive material has been found in the tissues of both the mother and the conceptus.

Teratology

This group is concerned with the environmental agents that cause birth defects in mammals. A large part of this group's effort is devoted to the development of an Environmental Teratology Information Center (ETIC), providing the biomedical community access to all publications that contain data pertinent to the teratogenic potential of environmental agents or conditions to which man is exposed. This program will help in defining priorities for future investigations and in prevening unplanned duplication of research. The first block

of information is now available on-line as a part of the National Library of Medicine's ToxLine.

Modern man is exposed continuously to a multitude of physical as well as chemical and infectious stresses. Most of these agents have not been tested for their teratogenic potential. In collaboration with scientists of the Laboratory of Environmental Biophysics, scientists have exposed mice to jet engine and "sonalert" noise. More than 3500 women of child-bearing age in the United States alone are currently being exposed to this type of noise at up to 130 dB. These types of noise, either singly or in combination, have not been found to cause teratogenicity in mice.

Simultaneous exposure to two or more chemicals represents a more realistic environmental exposure; and the toxicity of combined chemicals might be greater than their additive toxicities. In this regard, carbaryl, a widely used insecticide, was administered to the mouse alone and in combination with piperonyl butoxide or phenobarbital. No positive teratogenicity was detected.

In other studies, the administration of carbon monoxide with ethanol or with sulfur dioxide did not result in a teratogenic response in either rabbits or mice. In another project, scientists in this group and contractors at the Research Triangle Institute have detected teratogenicity following oral administration of polychlorinated biphenyls to mice. As part of the U.S.-U.S.S.R. Cooperative Program, a scientist in this group studied the teratogenic potential of the insecticide Dipterex in the rat, mouse, and hamster. Dipterex was teratogenic in all three species when given three times daily by gavage.

Molecular Embryology

This workgroup is concerned with defining and analyzing certain molecular aspects of the differentiation process. In particular, research concentrates on analyzing patterns of gene expression at the transcriptional and translational level in the model embryoid body/teratoma system (OTT 6050) in the mouse. In this system, the embryoid bodies, which consist of an outer ring of endoderm and an inner mass of embryocarcinoma cells, grow as an ascites in mice. Frequently, embryoid bodies "implant" on the liver, spleen, or mesentary and this stimulates the production of teratomas, predominately neuroepithelial.

By use of oligo-dT cellulose chromatography, the approach has been to isolate total poly(A)-containing RNA from embryoid bodies, teratomas, and the F9 embryocarcinoma cell line grown *in vitro*. These RNA preparations are then characterized with respect to poly(A) content, molecular size, and length of the poly(A) tract. Complementary DNA probes are prepared to these RNA preparations by use of reverse transcriptase, and nucleic acid hybridization techniques are used in analysis. The sequence and abundance of classes of these preparations are being defined. Results indicate that the RNA sequence populations in both embryoid bodies and teratomas can be divided into three classes, low, middle, and high complexity, with the tumors expressing 2-4 times more information in each RNA sequence class.

Another area of research involves the development of *in vitro* translation systems for use in isolating and purifying specific messenger RNAs. Preliminary data on *in vivo* patterns of protein synthesis suggest some of the major proteins synthesized in the embryoid bodies and teratomas. With this information, the potential messenger RNAs in the system can be identified and the *in vitro* translation system utilized to identify and purify these messenger RNAs. With particular messenger RNAs specific for the embryoid bodies or teratomas, specific complementary DNA probes can now be made for the analysis of gene expression in an *in vitro* chromatin/RNA polymerase system. Efforts are being made to define those molecules in chromatin involved in specific gene expression during the differentiation process.

Transplacental Toxicology

This group has demonstrated that prenatal exposure to DES adversely affects the reproductive capacity of the male and female offspring of mice. Such prenatal exposure results in a low incidence of female genital tract neoplasms including vaginal and uterine adenocarcinoma. Similar lesions could not be produced following prenatal exposure to the steroidal estrogen, 17^{-} β-estradiol. Differential fetal protein binding between DES and estradiol may help explain these results.

Scanning electron microscopy (SEM) of the genital tract of females exposed *in utero* has revealed surface ultrastructural abnormalities which may precede neoplastic changes. SEM may provide a tool for early detection of such changes. These studies are being correlated with observations on the role of steroid receptors in reproductive tissue alterations. In studies with male offspring from DES-treated mice, a high incidence of testicular and sperm abnormalities was detected. The fate of Mullerian duct remnants in DES-treated males is being determined, and the role of these tissues in prostatic lesions evaluated. The distribution, metabolism, and structure-activity relationships of DES in perinatal systems have been determined. The role of toxication-detoxication processes is

being evaluated in specific reproductive tract lesions. These studies emphasize the biological significance of potentially activated metabolites. These studies should aid in extrapolating these results to other classes of estrogenic environmental chemicals.

Developmental Pathology

This program seeks to determine the postnatal toxic effects on offspring of rats exposed to chemicals throughout gestation. Offspring are evaluated at various stages of development from newborn to geriatric. In addition to gross observations, the disposition of the chemical as well as effects on organ system function, immunological competence, learning and behavior are determined. Gross, histological, and subcellular pathology in offspring of treated and control females is analyzed. The offspring's adaptation ability and endurance capacity are also determined. Current studies demonstrate that methyl *n*-butyl ketone (MBK) and metabolites are present in both the fetal and maternal compartments in term pregnant rats. MBK-exposed females exhibit a 12–24 hr delay in parturition. Offspring of exposed animals, although similar at birth, show a progressive delay in development at all stages tested during the first six months. Biochemical changes and the mechanisms responsible need to be identified. Acute toxicity studies with methylglyoxal have shown that pregnancy protects against the toxic effects of the chemical. A possible explanation has been postulated. Other chemicals such as enflurane and ethchlorvynol have also been found to cause biochemical and behavioral changes during early postnatal development following gestational exposures. The characterization of potential organ pathology in offspring exposed *in utero* and the delineation of the total spectrum of postnatal toxicity as well as the mechanisms responsible for these effects are challenging areas of study in environmental health science.

Germ Cell Development

Group scientists are investigating the effects of environmental chemicals on germ cell toxicity. New approaches to reproductive toxicity testing are being explored and validated while other investigations are directed towards the elucidation of mechanisms of toxicity. Rat testes are used to study DNA repair synthesis and DNA breaks as an indicator for chemical mutagens, toxication-detoxication reactions of polycylic hydrocarbons in gonadal tissue, and toxic effects on reproductive function.

Alkaline elution analysis is a sensitive method for detecting DNA single strand breaks and/or cross-links in mammalian germ cells. Kinetics of alkaline elution of DNA single-strand breaks, stabilization of DNA, and/or increased DNA repair synthesis in germ cells after chemical exposure were found to be reliable indicators of toxicity. Recent investigations of testicular microsomal and soluble enzymes demonstrated that both aryl hydrocarbon activating and deactivating enzymes were present. Developmental patterns of these enzymes in rat testes indicated that glutathione *S*-transferase activity in prepubertal rats was 3-4 times greater than that of the liver enzyme. In contrast, epoxide hydrase activities of both liver and testes were very low in prepubertal rats but dramatic increases were evident at the onset of puberty. *In vitro* perfusion of the rat testis with tritium labeled benzo[a]pyrene 4,5-oxide indicated that the active benzo[a]pyrene metabolite was quickly conjugated or converted to a dihydrodiol derivative. The rates of detoxication via these two pathways suggested first-order kinetics; the reactions were concentration-dependent. Furthermore, *in vitro* perfusion of testis provides a unique model system for the integration of molecular, biochemical and physiological data with pharmacokinetic parameters. This approach for the study of environmentally-induced alterations of male reproductive organs should serve as an important model for extrapolation to human problems.

Developmental Toxicology

This group is concerned with perinatal aspects of enzymology, endocrinology, and pharmacology. A multidisciplinary effort is utilized in attempts to identify sensitive biochemical indicators of developmental toxicity having clinical value and to understand the mechanisms responsible for alterations in normal enzyme development. Emphasis is placed on the hormonal regulation of enzyme development in control and treated animals and the role of altered hormone action in developing systems. Biochemical and morphological changes are correlated with the uptake, binding, metabolism, accumulation, and clearance of foreign compounds and hormones in whole animals and culture systems. Current interests center on liver, intestine, reproductive tract and the pituitary-hypothalamic axis.

The specific fetal and newborn tissue distribution of several chlorinated and brominated biphenyls has been studied. Pregnant rats were administered labeled PCBs during various gestation periods and levels of radioactivity measured in a broad spectrum of maternal and fetal tissues and fluids. One surprising finding was that glucuronides of hydroxylated PCBs accumulate dramatically in fetal intestine

but these levels were rapidly depleted following birth. 2,4,5-2′,4′,5′-hexachlorobiphenyl (6-CB), which does not accumulate in the fetal intestine, is secreted in the milk although significant newborn tissue levels of this isomer also result from placental transfer. Significant levels of 6-CB were detected in brown fat immediately after birth. The brown fat is rapidly depleted, causing a redistribution of 6-CB to other tissues. 2,4,5-2′,4′,5′-hexabromobiphenyl (6-BB) crosses the placenta slowly but is secreted in high concentrations in milk. Further experiments are investigating the relative importance of placental transfer and milk secretion of 6-CB and 6-BB following treatment of female rats two weeks prior to mating.

The effect and mode of action of hormones and xenobiotics on the regulation of enzyme patterns during perinatal development is also being investigated. The postnatal development of rat liver histidase 5-α-reductase, 16-α-hydroxylase and several other organelle specific enzymes following perinatal exposures to diethylstilbestrol (DES), zeranol, the PCBs, testosterone and estradiol is being defined. Rat liver histidase and 5-α-reductase undergo a complex postnatal developmental course resulting in enzyme levels which are considerably higher in the adult female than the male. Recent experiments have demonstrated that prenatal exposure to DES does not alter hepatic histidase activities in immature male and female offspring and adult male offspring. However, histidase activities of adult female rats were decreased by 30% approaching activities of adult males. Thus, DES exerts an apparent programming effect on hepatic histidase following prenatal exposures. In contrast, the perinatal development of 5-α-reductase is unaffected by prenatal exposure to DES although a programmed response is evident when DES is administered during the first week after birth.

Another area of research focuses on hormonal and environmental factors affecting intestinal function and energy-producing processes during perinatal development to identify biochemical components indicative of normal and/or altered intestinal function. One study has shown that intestinal pyruvate dehydrogenase (PDH) is converted to its active form immediately after birth and that this conversion is correlated with concomitant changes in the mitochondrial redox state. Activation of PDH during the early postnatal period allows for increased utilization of pyruvate by the TCA cycle resulting in increased ATP production necessary for active transport to occur in the developing intestine. PDH activity and the activation process are highly sensitive to oral arsenic exposures. β-Glucuronidase (βG) activities are extremely high in the perinatal period and since fetal and newborn tissues often exhibit enzyme activities similar to those of tumor tissues, newborn intestinal isozyme profiles were compared to those of normal adult rats and rats exposed to dimethylhydrazine, a specific colon carcinogen. Preliminary results indicate that βG may provide an early enzyme indicator for intestinal carcinogenesis. An intestinal primary culture system formed by the isolation of tip and crypt cells is being developed and evaluated as a possible *in vitro* model for cellular differentiation, intestinal toxicity, and intestinal toxication-detoxication of environmental chemicals.

Biochemical Toxicology

Scientists in this workgroup study basic biochemical and physiological mechanisms through which environmental agents exert their toxic effects in mammalian tissues. The goal is to apply this information to designing rapid and effective test procedures for predicting toxicity in man.

Factors affecting hepatic heme synthesis and its use during mammalian development are being studied. Differences in regulatory and biochemical properties of several heme biosynthetic pathway enzymes in adult and fetal liver including those of α-aminolevulinic acid (ALA) synthetase, which is considered to be rate-limiting in this process, are being clarified. These studies also showed heme plays an important role in regulating synthesis of various mitochondrial proteins in fetal mammalian liver. This action is probably mediated through a direct stimulatory effect of heme on the synthesis of apocytochrome oxidase and, perhaps, other hemoproteins which comprise integral portions of the mitrochondrial structure. Because of the important regulatory role played by heme during fetal hepatic development, it is likely that late gestation may be a period of enhanced susceptibility to effects of environmental agents which selectively interfere with heme biosynthesis or its utilization. Studies are currently in progress to assess effects of prenatal exposure to hematotoxic agents on the postnatal development of hepatic hemoprotein function.

Other scientists are attempting to define the early ultrastructural and biochemical effects of heavy metals and other environmental agents on mammalian tissues to derive early warning tests for predicting subtoxic human responses to such agents. Their studies include determining the correlation between subcellular morphologic observations and biochemical alterations in tissues of animals chronically exposed to low levels of toxic agents for various periods. In current studies, researchers are examining the toxicity of arsenic, cadmium, lead,

methyl and inorganic mercury, dioxin (TCDD), and vinyl chloride. Results of their experiments indicate that specific morphological and biochemical response profiles can be established which differentiate various metals and other agents in our multielement environment. Studies are currently in progress to use these profiles to develop test procedures that will permit identification of preclinical exposure to specific environmental substances in human populations.

Behavioral Toxicology

NIEHS is developing a behavioral toxicology program in an attempt to define methods for determining what environmental stresses produce behavioral alterations in mammals and lower species. As part of this effort, scientists are developing model test systems which simulate and predict behavioral toxicity in humans and are considering neurochemical mechanisms of behavioral toxicity. Some research in this area involves the neurotoxicological effects of hexabrominated biphenyl compounds in male and female rats and mice receiving various doses for periods up to six months. In another series of experiments, an attempt is being made to determine the various factors in the etiology of pica (the consumption of seemingly nonnutritive substances) and to validate the utility of pica as a behavioral assay of toxicosis. A third study investigates the consequences of inhibiting monoamine oxidase A and B on different aspects of behavior. This research has consequences with respect to several pesticides which are known to inhibit monoamine oxidase.

Prospective

In the coming year, the Laboratory of Environmental Toxicology will continue to expand its research activities in each of the areas described. It will develop a more systematic routine testing capability, as well as validate and improve test methods. Additionally, the laboratory will attempt to extend laboratory animal observations to the human population. In this effort, it will encourage an increasingly strong interaction between laboratory scientists, clinicians, and epidemiologists.